Last round of DCAM specimens set for launch

Space Shuttle-Mir Science Program (STS-89/91)

Biotechnology Program Office

Protein Crystal Growth Program

Hardware

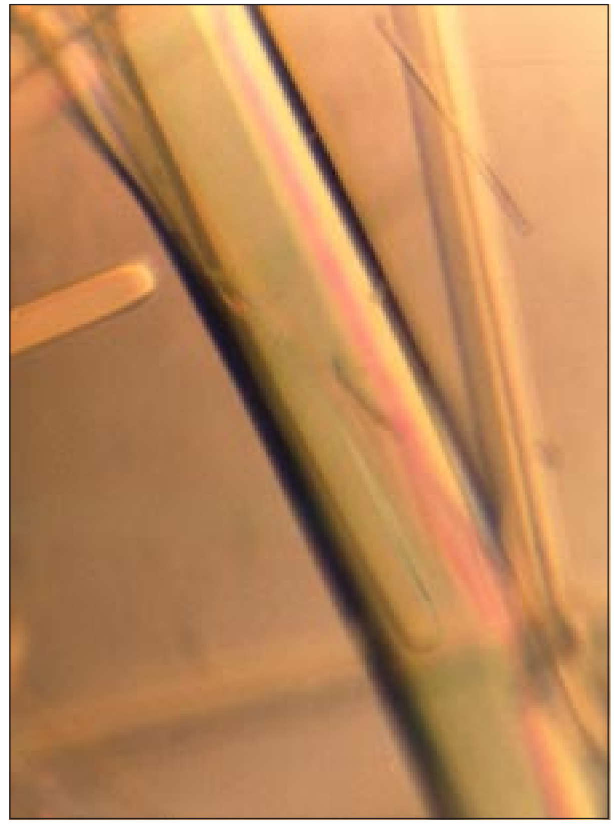
DCAM (Diffusion-controlled Crystallization Apparatus for Microgravity)

Experiments with the Diffusion-controlled Crystallization Apparatus for Microgravity (DCAM) aboard the Mir space station will conclude with a payload of six trays of cells to be carried by STS-89 in January 1998.

To date, with stays lasting as long as 6-months aboard Mir, DCAM has yielded dramatic results. Highlights include numerous large, spectacular crystals of the nucleosome core particle (shown at right), which regulates genetic activities in the nucleus of a cell. Another striking result was the growth of the largest T7 RNA crystal ever produced (0.7 x 0.8 x 2.0 mm in size). DCAM cells carrying triglycine sulfate (TGS) also yielded large crystals. TGS has been grown in space by other experiments, so these results will help in gauging the comparitive effectiveness of various microgravity processes.

DCAM's first flight, the second U.S. Microgravity Laboratory (USML-2) in 1995, verified the scientific validity of this approach to growing protein crystals. A full complement of six trays comprising 162 DCAM cells was carried to Mir in March 1996 and was returned to Earth on STS-79 which also installed a second set of DCAM trays. The STS-81 mission in January 1997 retrieved the second set of trays and installed a third set, retrieved by STS-85 in May 1997, as scientists continued to refine the mixtures and details used in this promising method. STS-91 will retrieve this DCAM set in May 1998.

Proteins are important, complex biological molecules which serve a variety of functions in all living organisms. Determining their three-dimensional structure will lead to a greater understanding of how they function in living organisms. Many proteins can be crystallized and their molecular structures determined through analysis of those crystals by X-ray diffraction. Unfortunately, some crystals grown in the 1-g environment of Earth have internal defects that limit or impair such analyses. As demonstrated on Space Shuttle missions since 1985, some protein crystals grown in space are larger, and more highly ordered, than the Earth-grown counterparts.



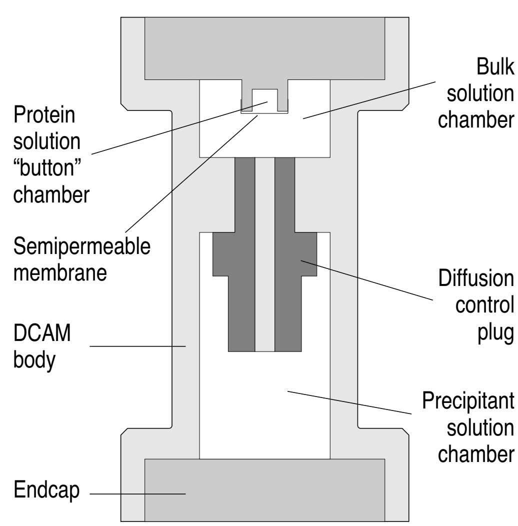
Crystal of nucleosome core particle.

DCAM was developed at Marshall Space Flight Center to grow protein crystals by a special diffusion process. The principal investigator is Dr. Daniel Carter of New Century Pharmaceuticals.

▶ Diffusion-controlled Crystallization Apparatus for Microgravity (DCAM)

DCAM grows crystals by the dialysis and liquid/liquid diffusion methods. In both methods, protein crystal growth is induced by changing conditions in the solution containing the protein. In dialysis, a semipermeable membrane retains the protein solution in one compartment, while allowing molecules of precipitant to pass freely through the membrane from an adjacent compartment. As the precipitant concentration increases within the protein compartment, crystallization begins.

In liquid-liquid diffusion, a protein solution and a precipitant solution are layered in a container and allowed to diffuse into each other. This leads to conditions which may induce crystallization of the protein. Liquid-liquid diffusion is difficult on Earth



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because density and temperature differences cause the solutions to mix rapidly.

In the DCAM, a "button" covered by a semi-permeable membrane holds a small protein sample but allows the precipitant solution to pass into the protein solution. Exposure to the precipitant causes the protein to crystallize.

Each DCAM unit is a polycarbonate plastic container a little larger than a plastic can for 35 mm film. The inside is molded into two cylindrical chambers joined by a tunnel. The first chamber, which is smaller, contains a buffer / precipitant solution. The end cap for this chamber holds the protein sample in a "button" covered by a semi-permeable membrane. The larger chamber holds a precipitant solution which is usually more concentrated that that in the smaller chamber. The two chambers are joined by a plug filled with a porous material to control the rate of diffusion. The plug material is selected to be compatible with the protein solution, and its properties are set to match the rate at which the crystals are to grow.

The DCAM has no mechanical system. Diffusion starts on Earth as soon as the chambers are filled. However, the rate is so slow that no appreciable change occurs before the samples reach orbit one or two or even several days later. This also allows protein samples to stay aboard the shuttle in case of a launch delay. In other hardware, many samples must be replaced in the event of a postponement. Such an apparatus is ideally suited for long duration mission such as the International Space Station and Mir.

Principal elements of a DCAM cell are shown here: assembled DCAM (top), the DCAM main body (left), and the end caps (center and right). Note that the fuse is visible in the assembled cell and the "button" is in the center of the cap at right.

STS-89 will carry 162 DCAM units mounted in 3 x 9 arrays on six trays stored in a locker in the Shuttle middeck (the same as the array now aboard Mir). Upon arrival at Mir, the DCAMs will be transferred to one of Mir's modules and mounted in a quiet area where crystallization will take place. Three of the six trays will be mounted so they can be photographed as crystals form. After the retrieval and return to Earth by STS-92 in May 1998, the samples will be analyzed.

Candidate samples

Protein samples for the crystallization in space are selected by a committee chaired by the PCG project scientist at MSFC. Samples are then evaluated and approved by NASA toxicology and safety offices. As a point of comparison, the molecular masses of proteins range from about 890 to 2,200 times that of ordinary sugar, a relatively simple organic compound which is easily crystallized. Candidate DCAM proteins (and guest investigators) for the fourth DCAM-Mir mission include:

Human Antithrombin III controls blood coagulation in human plasma. Its importance is underscored by the occurrence of severe thrombotic disorders including deep vein thrombosis, pulmonary embolism, and cerebral infarction in subjects with antithrombin mutations. Antithrombin is commonly given to patients suffering thrombotic crises of the shock syndromes. *Investigator:* Dr. Mark R. Wardell, Washington University School of Medicine, St. Louis, Mo.

Lysozyme is used a protein model to document the effects of microgravity on crystal growth. *Investigators*: Dr. Daniel C. Carter, New Century Pharmaceuticals, Huntsville, Ala., Dr. Franz Rosenberger and Dr. Bill Thomas, University of Alabama in Huntsville.

Nucleosome core particles have important roles in the regulation of gene expression, particularly in the expression of genes transcribed by RNA polymerase III. The nucleosome is the basis for organization within the genome by compacting DNA within the nucleus of the cell and by making selected regions of chromosomes available for transcription and replication. *Investigator*: Dr. Gerard J. Bunick, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Outer surface glycoprotein of the hyperthermophile Methanothermus fervidus lets M. fervidus live under environmental extremes, like high temperature, low-pH value, or high salt concentration. Elucidation of the crystal structure of this glycoprotein, which is directly exposed to the environment, may provide important information on the survival of these unusual microorganisms. *Investigator*: Dr. Jean-Paul Declercq, Université Catholique de Louvain, Belgium.

Serum Albumin, a key ingredient in blood plasma, is crucial to the transport of drugs and other chemicals throughout the body. *Investigator*: Dr. Daniel Carter, New Century Pharmaceuticals, Huntsville, Ala.

HK-Gro EL Complex is used in fundamental virus structure and function studies. *Investigator*: Dr. John Rosenberg University of Pittsburgh.

Ferritin and Apoferritin is used in fundamental biochemistry and crystal growth model systems. *Investigators*: Dr. Franz Rosenberger, Dr. Bill Thomas, University of Alabama in Huntsville, Dr. Daniel C. Carter, New Century Pharmaceuticals, Huntsville, Ala.

Bacteriorhodopsin is used in fundamental biochemistry studies. *Investigator*: Dr. Gottfried Wagner Justus-Liebig-University, Germany

Ferrochelatase is important to biomedical and biochemical applications. *Investigators*: Dr. B.C. Wang and Dr. Harry Dailey University of Georgia.

Notes